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14. ABSTRACT

The main objective of our studies is to elucidate the mechanisms by which PKCε, in conjunction with Pten loss, lead to malignant transformation and metastasis, through an autocrine mechanism that involves the release of the chemokine CXCL13. During the first year we acquired new evidence that CXCL13 levels are elevated in prostate cancer cells and that PKCε is causally associated with the elevated production and release of this chemokine. We also initiated studies to dissect the signaling mechanisms that mediate CXCL13 induction. We took advantage of a cellular model that we generated in our laboratory in which PKCε was overexpressed using a lentivirus in a Pten-deficient background. We also remediated the issue of loss of stable PKCε expression in prostate epithelial cell lines by generating a new PKCε lentivirus. Our research may impact on our understanding of the molecular mechanisms of prostate tumorigenesis, and may have significant prognostic and therapeutic implications.

15. SUBJECT TERMS

PKCε, Pten, CXCL13, CXCR5, proliferation, migration, tumorigenesis, metastasis, CXCL13 promoter, transcriptional activation, autocrine loop, mouse models.

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1. INTRODUCTION

This funded application focuses on the oncogenic kinase PKCε as a driver of prostate cancer progression. It is known that PKCε is overexpressed in human prostate tumors, however the significance of this altered expression and a potential causative role in the progression of prostate cancer is far from being understood. In our laboratory we generated a prostate-specific transgenic PKCε mice (PB-PKCε), which develops preneoplastic prostate lesions (PINs) with elevated phospho-Akt levels. Interestingly, when we expressed PKCε as a transgene in a background of haplodeficiency for the tumor suppressor Pten (PB-PKCε;$Pten^{+/-}$ mice), adenocarcinomas with invasive features appear in the prostate. In the search for mechanisms involved in this phenotype, we found that overexpression of PKCε and Pten loss in prostate epithelial cells strongly induce in a synergistic manner the expression of CXCL13, a chemokine that acts as the ligand of the CXCR5 receptor. The hypothesis that we wish to test is that PKCε overexpression and Pten loss activate individually and in a synergical manner an autonomous autocrine tumorigenic/metastatic loop that is mediated by CXCL13. We also hypothesize that PKCε is a CXCL13:CXCR5 effector that contributes to positively amplify this oncogenic response. The purposes of this investigation are: *a)* to establish the relevance of the CXCL13:CXCR5 axis *in vivo*, *b)* to determine the mechanisms behind the induction of CXCL13 by PKCε, and *c)* to establish the relevance of the PKCε/Pten-CXCL13 association in human prostate tumors. As Pten loss is one of the most common genetic alterations in human prostate cancer and PKCε overexpression is also found in most prostate tumors, we anticipate learning novel fundamental concepts about their contribution to prostate cancer progression. Our research should have significant mechanistic, prognostic and therapeutic implications for prostate cancer as a disease.

2. KEYWORDS

Prostate cancer, PKCε, Pten, CXCL13, CXCR5, Akt, proliferation, migration, CXCL13 promoter, transcriptional activation, tumorigenesis, metastasis, autocrine loop, cell lines, mouse models.

3. ACCOMPLISHMENTS

a. What were the major goals of the project?

The Specific Aims proposed for this application were as follows:

- Aim 1: To establish the relevance of the CXCL13:CXCR5 axis *in vivo*.
- Aim 2: To determine the mechanisms behind the induction of CXCL13 by PKCε.
- Aim 3: To establish the relevance of the PKCε/Pten-CXCL13 association in human prostate tumors.

For the first year, main tasks according to the SOW were as follows:

- To establish cellular models for inducible depletion of CXCL13 and CXCR5 in CaP-PKCε cells (Months 1-3).
- To assess the effect of CXCL13 and CXCR5 inducible silencing on the tumorigenic activity of CaP-PKCε cells (Months 3-8).
- To assess the effect of CXCL13 and CXCR5 inducible silencing on the metastatic activity of CaP-PKCε cells (Months 8-14).

b. What was accomplished under these goals?

During the first year, we obtained substantial data supporting the concept that the CXCL13:CXCR5 axis plays a role in prostate cancer progression. One of our first goals was to establish CaP-PKCε cell lines in which the expression of either CXCL13 or CXCR5 has been silenced using RNAi. Two cell lines had been generated in our laboratory, which were derived from prostate epithelial cells isolated from Pten knock-out mice, and engineered to overexpress PKCε (CaP2-PKCε and CaP8-PKCε). Both cell lines behave similarly, and as indicated in the preliminary data of our proposal, they display enhanced proliferation and motility rates, form colonies in soft agar, acquire an invasive phenotype, and most importantly, they form tumors upon inoculation into nude mice. On the other hand, cell lines in which either Pten was deleted or PKCε was overexpressed, were

unable to form tumors in nude mice. We concluded that PKCε overexpression and Pten loss cooperate for the development of prostate cancer. We recapitulated these data using genetically engineered mouse models that were developed in our laboratory, as presented in the preliminary data of our original application.

During the course of our research, unexpectedly we encountered a problem that took several months to solve: the CaP-PKCε cell lines loss the overexpression of PKCε upon multiple passages in culture, despite the fact that PKCε was expressed in a "stably" manner using a lentivirus. The reason for this loss in PKCe overexpression remains unknown. Several approaches were undertaken in order to troubleshoot this problem, including modifiying the selection conditions (blasticidin concentration) and re-infection with the PKCε lentivirus that we had previously generated in our laboratory. Unfortunately, none of these strategies worked, and this led to a significant delay in our research, primarily because many experiments depended on this cell line. Moreover, a main goal included the generation of CXCL13- and CXCR5-deficient CaP-PKCε derivatives for their subsequent characterization in tumorigenic and metastatic assays in nude mice.

In order to remediate this problem, we opted for generating a new PKCε lentivirus, as the old lentivirus failed to confer PKCε overexpression upon infection of a range of cell lines. Engineering the PKCε lentivirus commenced by PCR amplifying the full-length murine PKCε cDNA using oligonucleotides containing *Mun*I (N-terminus) and *Nhe*I (C-terminus) restriction sites. The resulting PCR product was ligated into the corresponding sites located in the entry pENTR 3C dual Selection vector (Invitrogen). An LR recombination reaction was then performed to transfer PKCε from the entry pENTR 3C vector into the pLenti6/V5-DEST lentiviral expression vector with blasticidin resistance gene (Invitrogen, Carlsbad, CA). The resulting PKCε insert was sequenced in its entirety on an Applied Biosystems DNA sequencing machine. Lentiviral particles were generated using HEK293T cells and the ViraPower Packaging System (Invitrogen). Afterwards, P8 and CaP8 cells were stably transduced with the PKCε lentivirus and subsequently subjected to blasticidin selection (3 μg/ml) for 10 days. Blasticidin-resistant P8-PKCε and CaP8-PKCε clones were isolated and screened for PKCε expression by immunoblotting with a specific anti-PKCε antibody. As shown in **Fig. 1**, we succeeded in establishing again CaP8-PKCε cells (Pten negative), as well as the corresponding P8-PKCε cell line (Pten positive). We are now again in a good position to establish the CXCL13- and CXCR5-depleted cell lines required for the project as well as their phenotypic characterization.

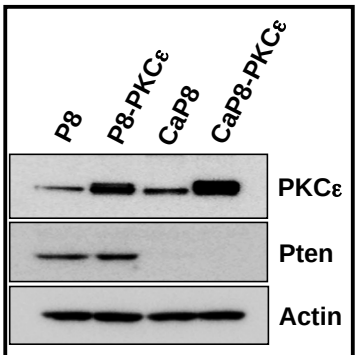

Fig. 1. Generation of cell lines. A PKCε lentivirus generated in our laboratory was used to infect P8 (Pten +) or CaP8 (Pten -) prostate epithelial cells. Stable cell lines were selected with blasticidin.

Characterization of signaling pathways responsible for CXCL13 induction:

While we were generating again the PKCε overexpressing cell lines, we decided to pursue experiments with the last batches of Pten-depleted cells that still overexpress PKCε available, or by using other means to achieve PKCε overexpression (adenoviral delivery), studies that would facilitate tasks for the subsequent years of this grant (particularly those of Specific Aim 2). Specifically, we initiated studies to establish the signaling pathways involved in CXCL13 induction by PKCε.

First, to determine if a causal relationship exists between PKCε levels and CXCL13 induction in prostate epithelial cells, we transiently overexpressed PKCε in parental (P8) cells using increasing multiplicities of infection (1-10 pfu/cell) of a PKCε adenovirus (AdV) (**Fig. 2a**). As shown in **Fig. 2b**, a significant induction in

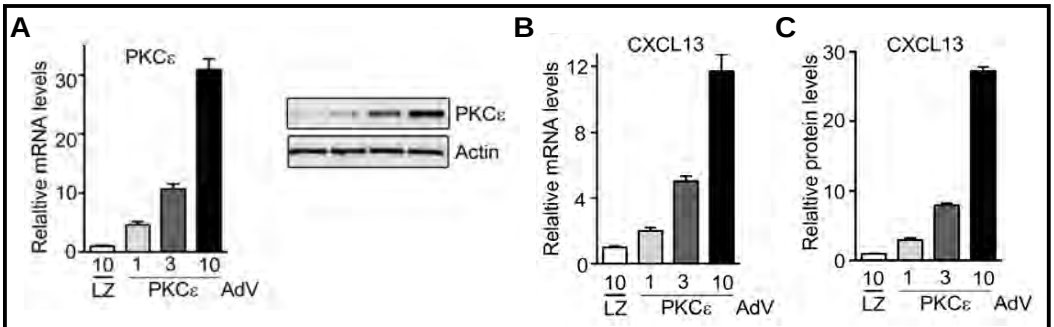

Fig. 2. PKCε overexpression induces CXCL13 in prostate epithelial cells. P8 cells were infected with different multiplicities of infection (1-10 pfu/cell) of PKCε AdV or LacZ AdV (*LZ*) as a control. *Panel A. Left*, PKCε mRNA levels, as determined by Q-PCR; *right*, Western blot analysis for PKCε expression. *Panel B.* CXCL13 mRNA levels, as determined by Q-PCR. *Panel C.* CXCL13 protein levels in the culture medium, as determined by ELISA. Data were expressed as relative to control (*LZ*) and represent the mean +/- S.D. of triplicate samples. Similar results were observed in 2 additional experiments.

CXCL13 mRNA levels was observed that was proportional to the levels of PKCε overexpression. Moreover, PKCε overexpression led to elevated protein levels of CXCL13 in the culture medium, as determined by ELISA (**Fig. 2c**). In addition, the requirement of PKCε for CXCL13 expression was determined in established prostate cancer cell lines (PC3 and DU145 cells). We found that silencing of PKCε expression in these prostate cancer cell lines using two different RNAi duplexes considerably reduced CXCL13 mRNA levels as determined by Q-PCR (**Fig. 3a**) and CXCL13 protein levels in the culture medium as determined by ELISA (**Fig. 3b**). Together, these observations argue for a stringent control of CXCL13 expression by PKCε in prostate cancer cells.

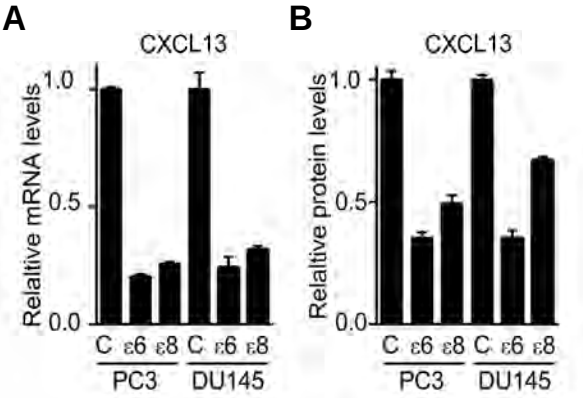

Fig. 3. PKCε RNAi depletion from prostate cancer cells reduces the expression and release of CXCL13. Prostate cancer cells (PC3, DU145) were transfected with PKCε RNAi duplexes (ε6 or ε8). After 48 h, CXCL13 mRNA levels were determined by Q-PCR (*Panel A*) or CXCL13 protein levels were determined in the culture medium by ELISA (*Panel B*). Data were expressed as relative to control cells transfected with a non-target control RNAi (*C*). Data were expressed as relative to control and represent the mean +/- S.D. of triplicate samples. Similar results were observed in an additional experiment.

CXCL13 mRNA levels were measured in human prostate cancer cells, and a significant up-regulation relative to immortalized non-transformed RWPE-1 cells was found (**Fig. 4a**). Accordingly, CXCL13 protein levels in the culture media were significantly higher in prostate cancer cell lines relative to RWPE-1 cells (**Fig. 4b**). This effect was prominent in the most aggressive, androgen-independent cell lines (LNCaP-abl, PC3, PC3-ML and DU145 cells), which in all cases display PKCε up-regulation.

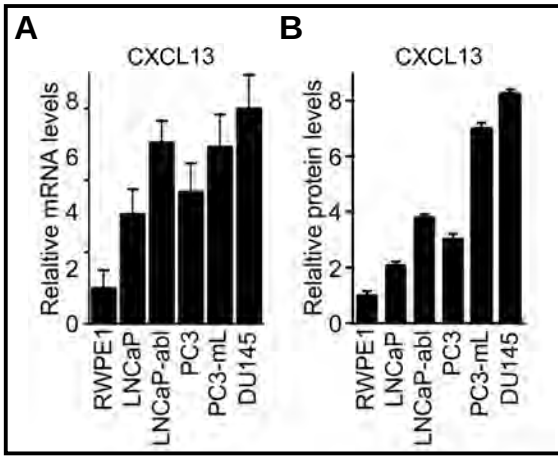

Fig. 4. Elevated CXCL13 expression in prostate cancer cells. *Panel A.* CXCL13 mRNA levels were determined by Q-PCR in a panel of cell lines that include normal "immortalized" (RWPE1) and prostate cancer cells. *Panel B.* CXCL13 protein levels were determined by ELISA in the culture medium of the different cell lines. Data were expressed as relative to RWPE1 cells and represent the mean +/- S.D. of triplicate samples. Similar results were observed in 2 additional experiments.

In initial experiments related to Aim 2, we explored the possibility that the NF-κB pathway was involved in CXCL13 induction driven by PKCε overexpression and Pten loss. Based on our previous publication (Garg et al., *JBC* 287:37570-37582, 2012), it became clear that the NF-κB pathway is regulated by PKCε in prostate cancer cells. In addition, bioinformatics analysis (not shown) revealed that a number of components of the non-canonical NF-κB pathway may be associated to PKCε. Indeed, we found that CaP8-PKCε cells display elevated levels of phospho-IKKα, suggesting hyperactivation of this pathway as a consequence of PKCε overexpression and Pten loss. An activated status for IKKα could be also detected in P8-PKCε cells, reinforcing the idea that even in the presence of Pten, PKCε overexpression is sufficient to activate the NF-κB pathway. Notably, CaP8-PKCε cells display elevated levels of RelB and NIK, signaling molecules associated with the non-canonical NF-κB pathway (**Fig. 5**).

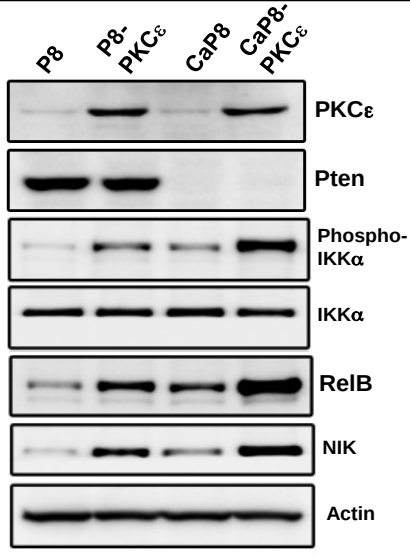

Fig. 5. Hyperactivation of non-canonical NF-κB signaling in prostate epithelial cells with PKCε overexpression and Pten loss. Western blot analysis for the different cell lines was carried out using specific antibodies.

To establish a link between the NF-κB pathway and CXCL13 expression, we used two different approaches. First, we used a NF-κB super-repressor ($I\kappa B\alpha^r$). Expression of the super-repressor significantly

reduced CXCL13 mRNA levels from CaP8-PKCε cells (**Fig. 6a**). In addition, we used RNAi to silence NIK and IKKα from CaP-PKCε, and found that silencing these key kinases of the non-canonical NF-κB pathway leads to a significant reduction of CXCL13 mRNA levels. On the other hand, CXCL13 mRNA levels were not affected upon silencing of IKKβ (**Fig. 6b**). These preliminary studies clearly suggest a link between the NF-κB, an effector of PKCε in prostate cancer cells, and the production of the chemokine CXCL13.

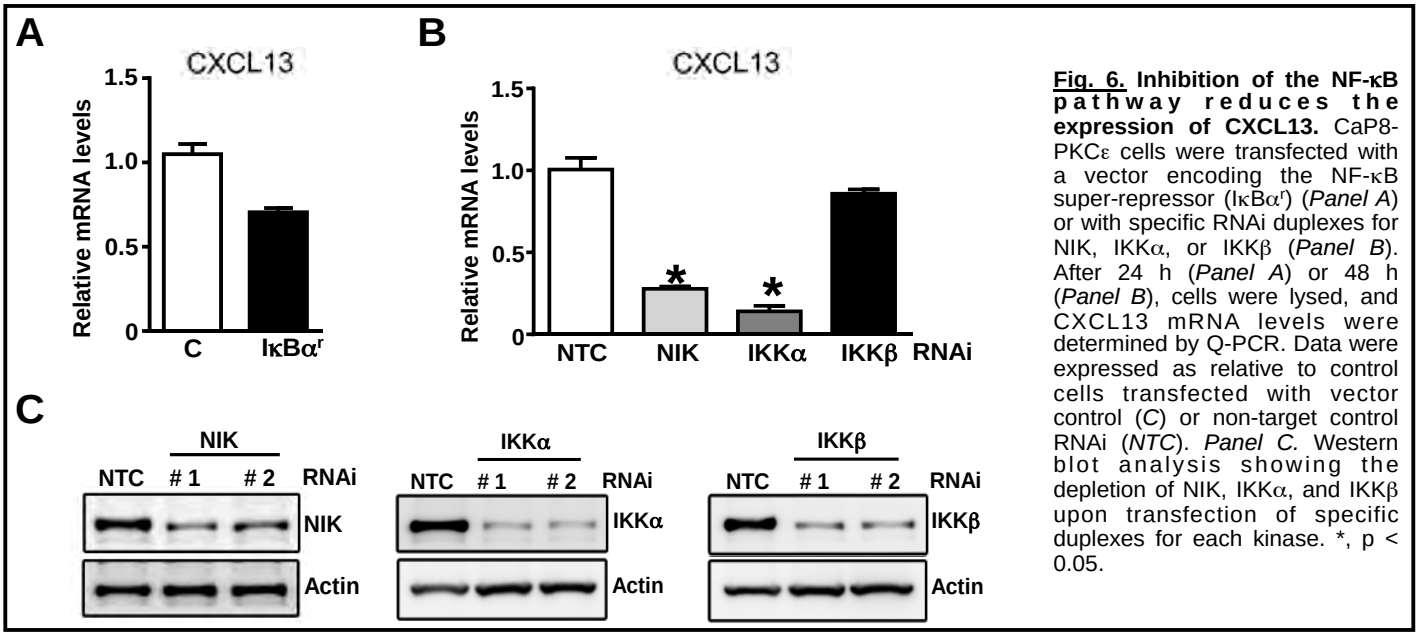

Fig. 6. Inhibition of the NF-κB pathway reduces the expression of CXCL13. CaP8-PKCε cells were transfected with a vector encoding the NF-κB super-repressor (IκBα^r) (*Panel A*) or with specific RNAi duplexes for NIK, IKKα, or IKKβ (*Panel B*). After 24 h (*Panel A*) or 48 h (*Panel B*), cells were lysed, and CXCL13 mRNA levels were determined by Q-PCR. Data were expressed as relative to control cells transfected with vector control (*C*) or non-target control RNAi (*NTC*). *Panel C.* Western blot analysis showing the depletion of NIK, IKKα, and IKKβ upon transfection of specific duplexes for each kinase. *, $p < 0.05$.

Our preliminary results strongly support the hypothesis that PKCε overexpression activates an autonomous autocrine loop that involves the release of the chemokine CXCL13. CXCL13 release is also triggered by Pten loss (hyperactivation of the PI3K/Akt pathway), and is markedly enhanced in PKCε overexpressing/Pten deficient cells. Furthermore, we hypothesize that PKCε is a CXCR5 effector that positively amplifies this "vicious cycle". Having herewith confirmed that PKCε up-regulates CXCL13 expression, we next speculated that the enhanced production of this chemokine contributes to phenotypes driven by PKCε overexpression. In a time-course experiment, we established that CXCL13 levels in the medium peak at 16 h following CaP8-PKCε cell seeding (**Fig. 7**). We speculated that conditioned media (CM) collected from prostate epithelial cells overexpressing PKCε and/or Pten depleted confers a migratory response through the CXCL13/CXCR5 axis. CM from P8, P8-PKCε, CaP8, and CaP8-PKCε cells were collected at 16 h and assessed for their "pro-motile" activity when added to naïve P8 cells. We found that the activity of the different CM varied as CM-CaP8-PKCε > CM-P8-PKCε ~ CM-CaP8 > CM-P8 cells (**Fig. 8**), which is in concurrence with the gradation of CXCL13 production from each cell line (shown in our original proposal). We are currently determining if this pro-motile effect is causally related to the released CXCL13. In a preliminary experiment, we silenced CXCL13 expression in CaP8-PKCε cells, and a marked reduction in CXCL13 mRNA levels was observed (data not shown). Therefore, it is possible that an autocrine CXCL13:CXCR5 loop mediates effects driven by PKCε overexpression and Pten loss.

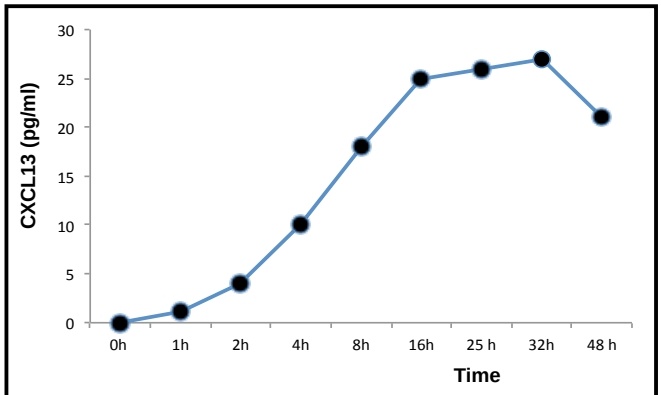

Fig. 7. Time-course for CXCL13 release to the conditioned medium (CM) from CaP8-PKCε cells. CXCL13 was determined in CM collected after the different times indicated in the figure. The mean of triplicate samples in a representative experiment is shown.

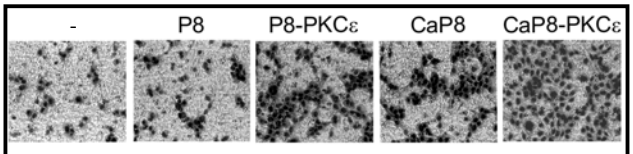

Fig. 8. Effect of conditioned medium (CM) from different cell lines on P8 prostate epithelial cell motility. CM was collected from cultures of different cell lines indicated in the figure, and then added to serum-starved parental P8 cells that were subsequently seeded in a Boyden chamber. Representative figures for migration after 16 h are shown in each case. -, no CM treatment.

c. What opportunities for training and professional development has the project provided?

Nothing to report.

d. How were the results disseminated to communities of interest?

An abstract was presented at the 106th Annual Meeting of the American Association for Cancer Research: Garg, R., Blando, J., Perez, C.J., Abba, M., Benavides, F., and Kazanietz, M.G. Protein kinase C ε cooperates with Pten deficiency to regulate NF-κB pathway in prostate cancer progression. Proceedings of American Association for Cancer Research 106th Annual Meeting. Cancer Res. 75 (15 Supplement): 799 doi:10.1158/1538-7445.AM2015-799 (2015). Honored for AACR-Aflac, Inc. Scholar-in-Training Award.

4. IMPACT

a. What was the impact on the development of the principal discipline(s) of the project?

The identification of the CXCL13:CXCR5 pathway as a mediator of the oncogenic/metastatic phenotype of prostate cancer cells is highly significant. CXCL13 and CXCR5 have been widely implicated in B cell migration, lymph node development and lymphomagenesis, and serum CXCL13 is a biomarker for many autoimmune diseases, HIV infection and lymphomas. CXCL13 and CXCR5 are expressed in prostate cancer cell lines, and it has been established that CXCL13 activates Akt and Erk in prostate cancer cells and induces prostate cancer cell migration and invasion in a PI3K-dependent manner. Our studies linking PKCε and Pten to CXCL13 production may impact on the understanding of the molecular mechanisms of prostate tumorigenesis, as they will shed light into the involvement of the CXCL13:CXCR5 pathway in transformation and invasiveness driven by defined alterations commonly occurring in prostate cancer patients. We believe that our studies should have significant prognostic impact. PKCε up-regulation is a well-established event in prostate cancer, and therefore we may find important correlations with other markers such as CXCL13 levels that could potentially be used as biomarkers of disease progression. One may expect that, if successful, our studies could also impact on the future development of CXCL13:CXCR5 inhibitors as anti-cancer agents, thus highlighting the translational significance of our studies.

b. What was the impact on other disciplines?

Although our research is primarily focused on prostate cancer, it is conceivable that similar mechanisms involving a link PKCε/Pten/CXCL13 apply to other cancers. Indeed, PKCε overexpression and Pten loss are not only hallmarks of prostate cancer but they frequently occur in other cancer types. Therefore, one may expect that these two alterations potentially lead to hyperproduction of CXCL13 in a range of cancer cells. As an example, emerging information suggests the involvement of the CXCL13:CXCR5 axis in the progression of breast and colon cancer. One may speculate that if we succeed in deciphering the molecular mechanisms of the CXCL13:CXCR5 pathway activation in prostate cancer as well as establish its significance in prognosis and therapeutics, conclusions of our research may also apply to other cancer types.

c. What was the impact on technology transfer?

Nothing to report.

d. What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS

a. Changes in approach and reasons for change

As described above, we encountered problems in maintaining stable expression of PKCε in the CaP-PKCε cells, which caused a significant delay in the generation of additional cell lines and their phenotypic characterization. Importantly, as described above (see Fig. 1), we succeeded in fixing this problem and generating these cell lines again, therefore we expect that we can go back now to our original plan.

For the sake of completion within the allocated time-frame, one potential change that is under consideration is the use of a standard shRNA approach rather than an inducible approach, which would allow extensive

optimization. Indeed, in preliminary studies we have recently found that we could stably knock-down CXCL13 and CXCR5 in prostate cancer cells using specific lentiviruses. This may save us significant time in our research without affecting the overall conclusion from our studies.

b. Actual or anticipated problems or delays and actions or plans to resolve them

As indicated above, there was a delay in the experiments due to the issue of stably maintaining PKCε overexpression. We believe that the problem has now been solved, and that PKCε expression will not be lost in the cell lines that we have recently generated. We carefully determine that PKCε overexpression is not lost in the new cell lines generated, which is checked regularly by Western blot after the multiple passages.

c. Changes that had a significant impact on expenditures

The problems we encountered in our experiments and potential changes that may occur to fix them should not have any significant impact on expenditures.

d. Significant chages in use or care of human subjects, vertebrates, biohazards, and/or selecte agents.

Nothing to report.

6. PRODUCTS

An abstract was presented at the 106th Annual Meeting of the American Association for Cancer Research: Garg, R., Blando, J., Perez, C.J., Abba, M., Benavides, F., and Kazanietz, M.G. Protein kinase C ε cooperates with Pten deficiency to regulate NF-κB pathway in prostate cancer progression. Proceedings of American Association for Cancer Research 106th Annual Meeting. Cancer Res. 75 (15 Supplement): 799 doi:10.1158/1538-7445.AM2015-799 (2015). Honored for AACR-Aflac, Inc. Scholar-in-Training Award.

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

a. What individuals have worked on the project?

There are no changes with regards to personnel. Personnel includes Marcelo G. Kazanietz (P.I.), Michael Feldman (Co-investigator), Rachana Garg (Research Associate), and Cynthia Lopez-Haber (Technician).

b. Has there been a change in the active other supporr of the PD/PI(s) or senior key personnel since the last reporting period?

The P.I. has been awarded two R01 projects during the last year.

- R01-CA189765 (P.I.: Kazanietz), NCI, NIH (04/15-03/20)

"CXCL13: a mediator of prostate cancer progression". The overaall goals are to understand the regulation of CXCL13 by specific p110 PI3K isozymes and PKC isozymes. A potential contribution of stromal CXCL13 will be examined using immunocompetent mice and depletion of lymphocytes involved in CXCL13 production. In addition, we will establish the relevance of the CXCL13:CXCR5 axis in metastatic dissemination to the bone, using established mouse models. Mechanistic studies will be pursued to explore the potential relevance of Rac guanine nucleotide exchange factors (Rac-GEFs) in the motile/invasive phenotype driven by the PKCε-PI3K-CXCL13-CXCR5 pathway. The contribution of individual p110 isoforms to CXCL13 induction and their cooperation with PKCε will be studied. CXCL13 expression in lesions from PKCε transgenic mice will be determined. The proposed experiments do not overlap with those in current proposal.

- R01-ES026023-01 (P.I.: Kazanietz), NIEHS, NIH (07/15-06/20)

"Protein kinase C and lung carcinogenesis". The main objective in this application is the characterization of PKC isozymes as effectors of K-Ras and mediators of the actions of carcinogens in lung cancer models. There is no overlap with current proposal.

c. What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Not applicable.

9. APPENDICES

Presentation Abstract

Abstract Number:	799
Presentation Title:	Protein kinase C ε cooperates with Pten deficiency to regulate NF-κB pathway in prostate cancer progression
Presentation Time:	Sunday, Apr 19, 2015, 1:00 PM - 5:00 PM
Location:	Section 34
Poster Board Number:	1
Author Block:	Rachana Garg[1], Jorge Blando[2], Carlos J. Perez[3], Fernando J. Benavides[3], Marcelo G. Kazanietz[1]. [1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]MD Anderson Cancer Center, University of Texas at Austin, Austin, TX; [3]MD Anderson Cancer Center, University of Texas, Smithville, TX
Abstract Body:	Prostate cancer is one of the most commonly diagnosed malignancies and the second leading cause of cancer-related deaths among men in the United States. Protein kinase C epsilon (PKCε), a member of the PKC family of phorbol ester/diacylglycerol receptors, has emerged as an oncogenic kinase and shown to be up-regulated in human prostate cancer specimens. We recently demonstrated that PKCε is an upstream regulator of NF-κB activation in prostate cancer (JBC, 287:37570-37582, 2012) and that transgenic overexpression of PKCε in mice prostate under the control of the probasin promoter (PB-PKCε) leads to hyperplasia and PIN lesions but was insufficient to drive neoplastic changes (Cell Cycle, 10:268-277, 2011). Notably, when we intercrossed PB-PKCε mice with mice haploinsufficient for Pten, another common genetic alteration in human prostate cancer, the resulting compound mutant mice (PB-PKCε;Pten+/- mice) developed fully invasive adenocarcinoma with elevated NF-κB levels. In the present study, we aim to delineate the mechanism underlying the observed cooperativity between PKCε overexpression and Pten deficiency and to explore the consequences of this cooperativity on the transcription factor NF-κB signaling, a pathway known to be highly dysregulated in prostate tumorigenesis. To this end, we stably overexpressed PKCε in mouse prostate epithelial lines that are either heterozygous (P8) or homozygous (CaP8) for Pten deletion. We observed a striking synergism between PKCεε overexpression and Pten loss in conferring enhanced proliferative, migratory and invasive phenotype. Moreover, LPS or TNFα stimulation of these cells led to increased NF-κB activation as evident from the elevated IκBα phosphorylation, NF-κB nuclear translocation and transactivation of a NF-κB luciferase reporter. These effects were much more pronounced in CaP8 cells. Of note, PKCε overexpression and Pten loss also cooperates to augment levels of the NF-κB regulated gene, COX-2. Stable overexpression of PKCε and Pten depletion in "normal" immortalized RWPE1 cells also resulted in significant enhancements in TNFα-induced NF-κB activation and COX-2 induction. Furthermore, NF-κB inhibition by parthenolide significantly retarded the growth of CaP8-PKCε tumors in athymic nude mice. Overall, our results identify NF-κB as a mediator of PKCε oncogenesis in prostate cancer, particularly in the context of Pten loss.

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